Original Contribution

Brain oxidative stress in a triple-transgenic mouse model of Alzheimer disease

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ABSTRACT

Alzheimer disease (AD) is a neurodegenerative disease which is characterized by the presence of extracellular senile plaques mainly composed of amyloid-β peptide (Aβ), intracellular neurofibrillary tangles, and selective synaptic and neuronal loss. AD brains revealed elevated levels of oxidative stress markers which have been implicated in Aβ-induced toxicity. In the present work we addressed the hypothesis that oxidative stress occurs early in the development of AD and evaluated the extension of the oxidative stress and the levels of antioxidants in an in vivo model of AD, the triple-transgenic mouse, which develops plaques, tangles, and cognitive impairments and thus mimics AD progression in humans. We have shown that in this model, levels of antioxidants, namely, reduced glutathione and vitamin E, are decreased and the extent of lipid peroxidation is increased. We have also observed increased activity of the antioxidant enzymes glutathione peroxidase and superoxide dismutase. These alterations are evident during the Aβ oligomerization period, before the appearance of Aβ plaques and neurofibrillary tangles, supporting the view that oxidative stress occurs early in the development of the disease.

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Alzheimer disease (AD) is a neurodegenerative disease characterized by the presence of senile plaques mainly composed of fibrillar amyloid-β peptide (Aβ) [1] and neurofibrillary tangles (NFTs) composed of paired helical filaments (PHF) of hyperphosphorylated tau [2,3]. Plaques and tangles are present mainly in brain regions involved in learning and memory such as cortex and hippocampus. These affected regions typically exhibit synaptic and neuronal loss, with cholinergic and glutamatergic neurons being the most affected [4].

Whereas the majority of AD patients suffer from the sporadic form of the disease, there is an inherited familial form caused by rare mutations in the APP or PS1 gene [5]. However, the neuropathological features are shared by both sporadic and familiar forms. Aβ can accumulate through overproduction or decreased clearance, leading to neurotoxicity and cell death. Although the mechanisms through which Aβ exerts its toxicity remain unclear, it seems that oxidative stress plays an important role [6,7]. Aβ and oxidative stress are linked to each other because Aβ produces oxidative stress [8–11], and pro-oxidants, in turn, increase Aβ production [12,13]. Moreover, several antioxidants, namely vitamin E and melatonin, were shown to protect neurons from Aβ-induced toxicity [8–11]. Aβ-mediated oxidative stress can be due to either an increase in reactive oxygen species (ROS) production or a decrease in the endogenous antioxidants, namely, in the activity of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) and of nonenzymatic anti-oxidants such as vitamin E and GSH [14]. Elevated levels of oxidative stress markers, namely protein carbonyls, thiobarbituric acid-reactive substances (TBARS), 4-hydroxy-2-$trans$-nonenal (HNE), 8-hydroxy-2-deoxyguanine (8-OHdG), and 8-hydroxyguanine (8-OHG), have been found in AD brains [15,16] and it has been suggested that oxidative stress is an early event that contributes to AD pathology before the appearance of amyloid plaques [17,18].

Recently, Oddo and colleagues developed a new AD mouse model that harbors PS1 $_{M146 V}$, APP$_{Swe}$, and tau$_{P301L}$ mutations [19] and that progressively develops extracellular senile plaques, intracellular NFTs, and cognitive impairments [19–21]. Furthermore, this model is crucial to study the relationship between Aβ and tau pathologies. In fact, it

has been demonstrated that genetically augmenting tau levels and hyperphosphorylation in the 3×Tg-AD mouse has no effect on the onset and progression of Aβ pathology, suggesting that the link between Aβ and tau is predominantly if not exclusively unidirectional [22]. Moreover, Aβ immunotherapy reduces soluble tau and ameliorates behavioral deficits in old transgenic mice [23,24]. In addition, Billings and colleagues [25] have demonstrated that spatial training reduces Aβ and tau pathologies and amielorates the spatial memory decline.

Given the critical role that oxidative stress plays in the pathogenesis of AD, the present work was aimed at evaluating the extension of oxidative stress and the levels of antioxidant defenses in the 3×Tg-AD mouse that closely mimics AD progression in humans [19,20]. We have observed increased levels of oxidative stress, in particular, enhanced lipid peroxidation and decreased levels/activity of both enzymatic and nonenzymatic antioxidants, in these transgenic mice before the appearance of Aβ plaques and neurofibrillary tangles, supporting the view that oxidative stress occurs early in the development of the disease.

Experimental procedures

Materials

Reduced (GSH) and oxidized glutathione (GSSG), nitroblue tetrazolium (NBT), GPx and glutathione reductase (GRd), SOD, xanthine oxidase, hypoxanthine, *ortho*-phetaldialdehyde (OPT), *N*-ethylmaleimide (NEM), β-nicotinamide adenine dinucleotide phosphate reduced form (β-NADPH), and *tert*-butylhydroperoxide (*t*-BHP) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The Oxiselect HNE–His Adduct ELISA Kit was purchased from Cell Biolabs (San Diego, CA, USA). All the other chemicals were obtained from Sigma Chemical Co. or from Merck kgaA (Damstadt, Germany).

Transgenic mice and brain homogenate preparation

The derivation and characterization of triple-transgenic (3×Tg-AD) mice have been described previously [19,20]. Briefly, human APP cDNA harboring the Swedish mutation (KM670/671NL) and human four-repeat tau harboring the P301L mutation were comicroinjected into single-cell embryos of homozygous PS1 $_{M146V}$ knock-in mice. The PS1 mice were originally generated on a hybrid 129/C57BL6 background [26]. Age-and gender-matched nontransgenic and PS1 knock-in mice were used as controls. Non-Tg, PS1, and 3×Tg-AD mice were obtained from Dr. Frank LaFerla's laboratory at the Department of Neurobiology and Behaviour and Institute for Brain Aging and Dementia, University of California at Irvine. Brain cortices isolated from 3-to 5-month-old females were frozen and stored at –80 °C before being homogenized in 0.32 M sucrose, 1 mM EDTA, 10 mM Tris, pH 7.4. Protein content of brain homogenates was determined by using the Bio-Rad protein dye assay reagent.

Measurement of lipid peroxidation

The extent of lipid peroxidation in brain homogenates was determined by measuring TBARS and malondialdehyde (MDA). TBARS levels were quantified using the TBA assay [27]. Brain homogenates were diluted two times with 15% trichloroacetic acid, 0.375% TBA, 0.25 M HCl, and 0.015% 2,6-di-*tert*-butyl-4-methylphenol and boiled for 15 min. The samples were chilled on ice and centrifuged for 10 min at 95.5 g in an Eppendorf 5810R centrifuge. The absorbance of the collected supernatants was then measured at 530 nm using a microplate reader (Spectra Max Plus 384; Molecular Devices). The amount of TBARS formed was calculated using a molar extinction coefficient of $1.56 \times 10^5 M^{-1} cm^{-1}$ and expressed as nanomoles TBARS produced per milligram of protein. The MDA levels were determined by high-performance liquid chromatography (HPLC) [28], using a Gilson HPLC

apparatus with a reverse-phase column (RP18 Spherisorb, S5 OD2). The samples were eluted at a flow rate of 1 ml/min and detection was performed at 532 nm. The MDA content was calculated from a standard curve prepared using the thiobarbituric acid–MDA complex and was expressed as nanomoles per milligram of protein. As a measure of lipid peroxidation, the levels of hydroxynonenal–histidine (HNE–His) protein adducts were also quantified by using the Oxiselect HNE–His Adduct ELISA Kit (Cell Biolabs, Inc.). The quantity of HNE–His protein adduct in brain homogenates was determined using a standard curve containing known amounts of HNE–BSA (0-10 μg/ml).

Measurement of superoxide dismutase activity

The activity of SOD was evaluated using a spectrophotometric assay described by Flohé and Ötting [29]. After 2 min incubation of 100 μg of protein in 1.4 ml of phosphate buffer (50 mM K_2HPO_4 and 100 μM EDTA, pH 7.8) containing 200 μl 0.025 mM hypoxanthine, 66.7 μl Triton X-100, and 66.7 μl 0.1 mM NBT, the reaction was initiated by the addition of 2 μl 0.025 U/ml xanthine oxidase. The reduction of NBT was measured at 550 nm (V560 UV/Vis spectrophotometer) for 3 min, at 25°C against a blank prepared in the absence of hypoxanthine. The activity of SOD was calculated using a standard curve containing known amounts of SOD (0.25-2 U).

Measurement of glutathione peroxidase and glutathione reductase activities

GPx and GRd activities were determined spectrophotometrically at 340 nm by the analysis of NADPH oxidation [30,31]. The activity of GPx was measured after a 5-min incubation, in the dark, of 10 μl of each sample with 100 μl phosphate buffer (0.25 M KH_2PO_4, 0.25 M K_2HPO_4, 0.5 mM EDTA, pH 7.0), 100 μl 10 mM GSH, 100 μl 1 unit GRd, and 480 μl H_2O. Then, 100 μl 2.5 mM NADPH and 100 μl 12 mM *t*-BHP were added and the absorbance was measured at 340 nm (Jasco V560 UV/Vis spectrophotometer) for 5 min, with continuous stirring, against blanks prepared in the absence of NADPH.

For the determination of the activity of the GRd, 200 μl of each sample was incubated for 30 s with 1 ml phosphate buffer (0.2 M KH_2PO_4, 2 mM EDTA, pH 7.0), 100 μl 2 mM NADPH, and 700 μl H_2O. The reaction was initiated by the addition of 20 mM GSSG. After 3 min at 30°C with continuous stirring, the absorbance was measured at 340 nm (Jasco V560 UV/Vis spectrophotometer), against blanks in the absence of GSSG. Results were normalized for the amount of protein per sample.

Measurement of glutathione content

Brain levels of reduced and oxidized glutathione were measured using a fluorimetric assay, according to Hissin and Hilf [32]. Briefly, 1 mg of protein from the brain homogenates was rapidly centrifuged at 100,000 g (Beckman, TL-100 ultracentrifuge) for 30 min with 1.5 ml phosphate buffer (100 mM NaH_2PO_4, 5 mM EDTA, pH 8.0) and 0.5 ml 2.5% H_3PO_4 (v/v). GSH levels were measured after the addition of 100 μl of OPT (1 mg/ml in methanol) to 100 μl of the sample supernatant and 1.8 ml phosphate buffer and incubation at room temperature for 15 min. For GSSG determination, 250 μl of the supernatant was added to 100 μl of NEM (5 mg/ml in methanol) and incubated at room temperature for 30 min. Then, 140 μl of this mixture was incubated for 15 min with 100 μl OPT in 1.76 ml NaOH (100 mM). Finally, the fluorescence was measured at 420-and 350-nm emission and excitation wavelengths, respectively. The measurements were performed in a Perkin–Elmer Luminescence Spectrometer LS 50B. The GSH and GSSG levels were determined by comparison with linear standard curves containing known concentrations of GSH or GSSG (0-1 μg) and results were normalized for the amount of protein per sample.
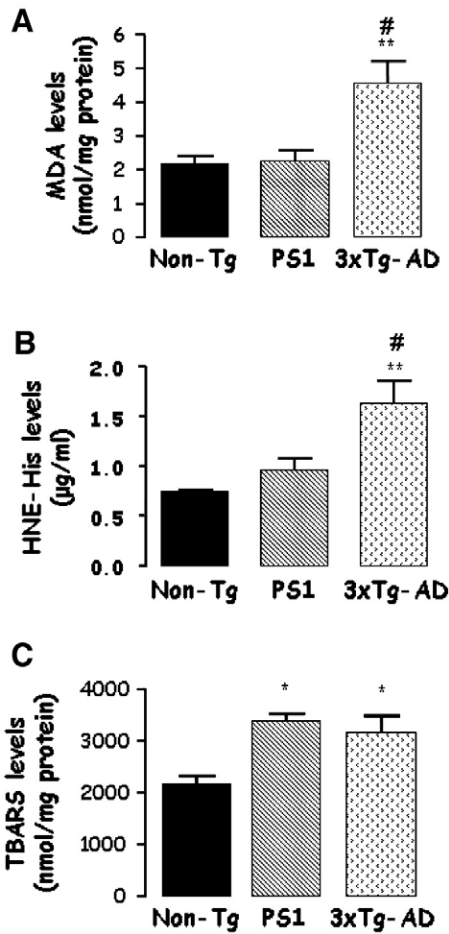
Fig. 1. Lipid peroxidation occurs in the brain of 3×Tg-AD mice. Brain homogenates were prepared from cerebral cortex of 3-to 5-month-old 3×Tg-AD mice and also age-matched PS1 and non-Tg mice and the extent of lipid peroxidation was evaluated by determining the production of (A) MDA, (B) HNE–His protein adduct, and (C) TBARS. Data, expressed as nanomoles per milligram of protein or microgram per milliliter, are means±SEM of the values from at least 10 animals. *$p < 0.5$, **$p < 0.01$, statistically significant compared with the control mice. #$p < 0.5$, statistically significant compared with PS1 mice.

Results

Lipid peroxidation is enhanced in the 3×Tg-AD mice

The extent of lipid peroxidation was evaluated by measuring the levels of MDA and TBARS, which are products of the oxidative modification of lipids [16,34]. HNE, another lipid peroxidation marker [16], structurally modifies proteins, forming stable adducts termed advanced lipid peroxidation end products. Because His residues are major targets [35], we have also quantified the levels of HNE–His protein adducts. Brain homogenates were prepared from the cerebral cortex of 3-to 5-month-old 3×Tg-AD mice and the levels of MDA, TBARS, and HNE–His were compared with those determined in age-matched PS1 mice and also in non-Tg animals. At this age the 3×Tg-AD mouse has not yet developed neither amyloid plaques nor NFTs [19,20]. The PS1 mice express mutant PS1 protein at normal physiological levels in the absence of endogenous wild-type mouse PS1 [26]. As shown in Fig. 1A an increase in MDA levels occurred in the brains of the 3×Tg-AD mice, but not in the PS1 mice. Similarly, the levels of HNE–His protein adduct are higher in 3×Tg-AD mice than in non-Tg and PS1 mice (Fig. 1B). A significant increase in the levels of TBARS was measured in both 3×Tg-AD and PS1 mice (Fig. 1C) compared with that determined in non-Tg littermates.
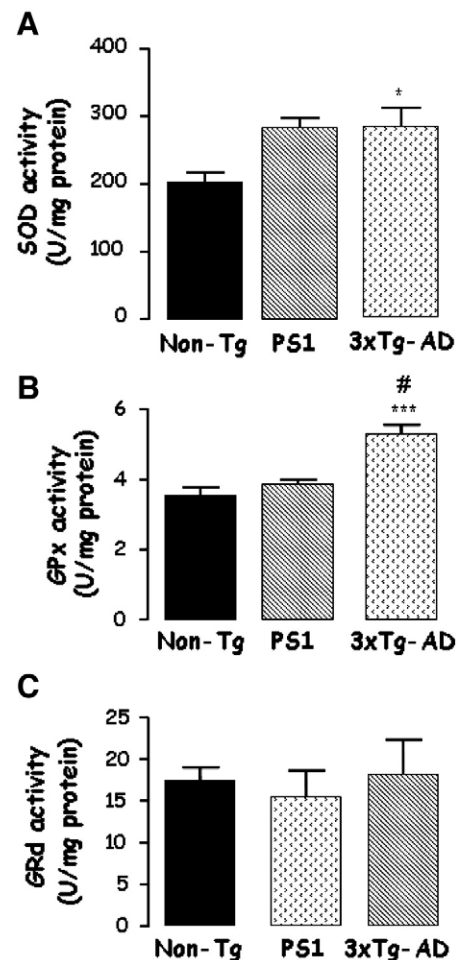
Extraction and quantification of vitamin E

Extraction and separation of vitamin E (α-tocopherol) from brain homogenates were performed by following a previously described protocol by Vatassery and Younoszai [33]. Briefly, 1.5 ml sodium dodecyl sulfate (10 mM) was added to 0.5 mg brain homogenate, followed by the addition of 2 ml ethanol. Then, 2 ml hexane and 50 μl of 3 M KCl were added, and the mixture was vortexed for about 3 min. The extract was centrifuged at 2000 rpm (Sorvall RT6000 refrigerated centrifuge) and 1 ml of the upper phase, containing *n*-hexane (*n*-hexane layer), was recovered and evaporated to dryness under a stream of N_2 and kept at -80°C. The extract was dissolved in *n*-hexane, and vitamin E content was analyzed by reverse-phase HPLC. A Spherisorb S10w column (4.6×200 nm) was eluted with *n*-hexane modified with 0.9% methanol, at a flow rate of 1.5 ml/min. Detection was performed by a UV detector at 287 nm. The levels of vitamin E were calculated as nanomoles per milligram of protein.

Statistical analysis

Data were expressed as the means±SEM of the indicated number of experiments. Statistical significance was determined by using one-way ANOVA followed by Tukey post hoc tests. The differences were considered significant for *p* values <0.05.

Fig. 2. The enzymatic activity of antioxidant enzymes is affected in the 3×Tg-AD mice. In brain homogenates prepared from 3-to 5-month-old 3×Tg-AD, PS1, and non-Tg mice the activity of (A) SOD and also of the enzymes of the glutathione redox cycle, (B) GPx and (C) GRd, was determined spectrophotometrically. Data, expressed as units per milligram of protein, are the means±SEM of the values from at least 10 animals. *$p < 0.5$, ***$p < 0.001$, statistically significant compared with the control mice. #$p < 0.5$, statistically significant compared with PS1 mice.
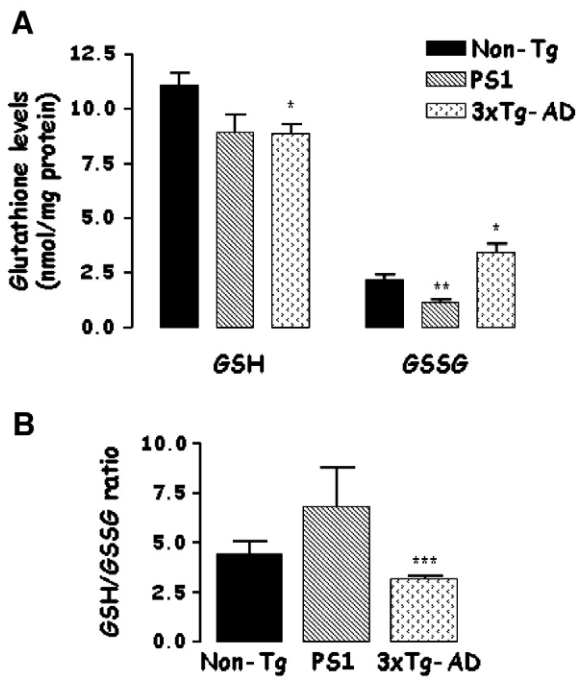
Fig. 3. GSH and GSSG levels and GSH/GSSG ratio are impaired in the 3×Tg-AD mice. Brain cortical homogenates were prepared from 3-to 5-month-old 3×Tg-AD, PS1, and non-Tg mice. (A) The levels of GSH and GSSG were determined using a fluorimetric assay and (B) GSH/GSSG ratio was calculated. Data, expressed as nanomoles per milligram of protein, are means±SEM of the values from at least 10 animals. *$p<0.5$, **$p<0.01$, ***$p<0.001$, statistically significant compared with the control mice.

The activity of antioxidant enzymes is increased in the brains of the 3×Tg-AD mice

SOD, GPx, and GRd are three enzymes involved in cellular protection against damage induced by oxygen-derived free radicals [14]. The activity of these antioxidant enzymes was measured in brain homogenates prepared from the cerebral cortex of 3-to 5-month-old 3×Tg-AD mice, age-matched PS1, and wild-type littermates. Unlike the PS1 mice, a significant increase in the activities of SOD (Fig. 2A) and of GPx (Fig. 2B) was observed in the brains of the 3×Tg-AD animals compared with the controls. The activity of GRd, which is involved in GSH recycling, was not affected in the PS1 or 3×Tg-AD (Fig. 2C).

Glutathione content is altered in 3×Tg-AD mice

GSH is one of the most relevant cellular nonenzymatic antioxidants [14]. In the 3×Tg-AD mice, GSH levels were significantly decreased with a concomitant increase of GSSG (Fig. 3A). As a consequence, the GSH/GSSG ratio determined in brain homogenates obtained from these mice was lower than that measured in the non-Tg littermates (Fig. 3B). On the other hand, in age-matched PS1 mice, the GSH levels were not significantly affected and GSSG deceased in a significant manner (Fig. 3A). As a result, GSH/GSSG ratio was not significantly changed in comparison with the non-Tg animals.

Vitamin E levels are decreased in the 3×Tg-AD mice

Vitamin E is the most effective lipid-soluble antioxidant that is able to block the lipid peroxidation chain reaction [36]. As depicted in Fig. 4, the vitamin E content in 3×Tg-AD mice brain homogenates was significantly decreased compared with the control animals. In PS1 transgenic animals, the levels of this nonenzymatic antioxidant were not significantly different from those determined in the controls.

Discussion

Extracellular senile plaques mainly composed of fibrillar Aβ [1] and NFTs composed of PHF of hyperphosphorylated tau [3] are the major neuropathological features of AD. Brain areas involved in learning and memory processes are reduced in size as a consequence of synaptic loss and neuronal death that seem to be associated with enhanced oxidative stress [4]. In fact, AD brains exhibit elevated levels of oxidative stress markers, namely protein carbonyls, TBARS, HNE, MDA, 8-OHdG, and 8-OHG [4,15,16,34]. HNE readily reacts with proteins and HNE–protein adducts have been found in AD brains [37]. More recent studies of patients with amnestic mild cognitive impairment, the earliest manifestation of AD, show similar patterns of oxidative damage [38]. These observations suggest that oxidative damage to critical biomolecules occurs early in the pathogenesis of AD and precedes pronounced neuropathologic alterations. Oxidative stress up-regulates BACE expression and activity [39] and increases Aβ levels [13]. On the other hand, several in vitro [8–10] and in vivo [11] studies have demonstrated that oxidative stress is involved in Aβ-induced toxicity, which is prevented by antioxidants, namely vitamin E and melatonin.

In the present study we evaluated oxidative stress in a triple-transgenic model of AD (3×Tg-AD). Epidemiologic studies have reported the higher incidence of AD in females [40–42], and Schuessel and colleagues [43] observed a gender-specific higher vulnerability in female AD patients toward oxidative stress. Taken together, these results led us to use female mice. The 3×Tg-AD mouse model develops senile plaques, NFTs, and cognitive impairments in an age-and region-dependent manner that closely mimics the human disease progression [19–21]. Intraneuronal Aβ is first detected in cortical brain regions, whereas the most extensive tau immunoreactivity is apparent in the CA1 region of the hippocampus, progressively affecting neurons in the cerebral cortex of older animals. Despite equivalent overexpression of human APP and tau, Aβ pathology precedes tau pathology by several months. Extracellular Aβ deposits in cortex are apparent by 6 months of age but tau alterations are not apparent before 12 months of age [19]. In this work, we addressed the hypothesis that oxidative stress is an early event in the progression of AD; therefore, we have used 3-to 5-month-old animals that have not yet developed neither Aβ nor tau pathologies. Even in the absence of these neuropathological hallmarks, an increase in the extent of lipid peroxidation, an oxidative stress marker, was observed. These results support other previous studies that demonstrate a negative correlation between oxidative damage and Aβ deposition in AD brain [44,45] and support that oxidative stress is an early event in the development of AD [17,18]. In the 3×Tg-AD mice, the oligomerization of Aβ starts between 2 and 6 months of age [24], suggesting that the oxidative stress observed in the 3-to 5-month-old mice can be initiated by oligomeric Aβ. Recently, De Felice and colleagues [46] demonstrated that soluble forms of the Aβ peptide, the amyloid-derived diffusible ligands, stimulate excessive formation of
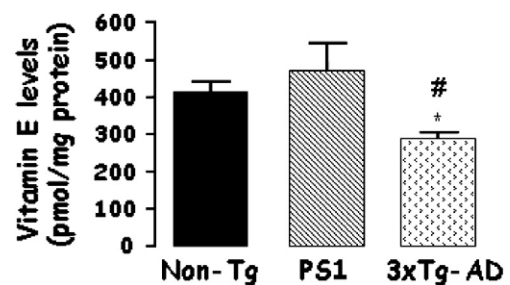

Fig. 4. The brain levels of the lipophilic antioxidant vitamin E are depleted in the 3×Tg-AD mice. Vitamin E (α-tocopherol) present in brain homogenates obtained from 3×Tg-AD, PS1, and non-Tg mice was measured by reverse-phase HPLC as described under Experimental procedures. *$p<0.5$, statistically significant compared with the control mice. Data, expressed as nanomoles per milligram of protein, are means±SEM of the values from at least 10 animals. #$p<0.5$, statistically significant compared with PS1 mice.
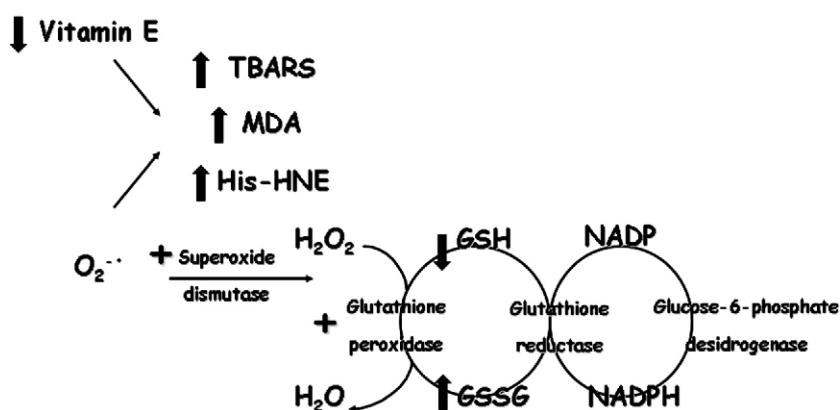
Fig. 5. Oxidative stress is increased in 3×Tg-AD mice. The decrease in nonenzymatic antioxidants such as vitamin E and GSH leads to lipid peroxidation, increasing the levels of MDA, HNE–His protein adducts, and TBARS. In the face of increased oxidative stress the activity of the antioxidant enzymes SOD and GPx is increased. The activity of GRd is not affected, leading to a further depletion of GSH levels.

ROS through a mechanism requiring *N*-methyl-D-aspartate receptor activation. The higher neurotoxicity exerted by soluble Aβ1-42 in comparison with fibrillar Aβ suggests that this event can be mediated by an increase in oxidative stress leading to cell death [47]. The fibrillar form of the peptide, being less pro-oxidant and cytotoxic, may preferentially induce toxicity by modulating BACE-1 expression and activity, increasing the amyloidogenic APP processing, and then resulting in a further accumulation of Aβ [48]. Soluble Aβ levels in Tg2576 mice have been directly correlated with increases in H_2O_2 [48], suggesting that soluble Aβ may be responsible for its production.

Oxidative stress can be due to either an increase in ROS production or a decrease in the activity of the antioxidant enzymes such as SOD and GPx or nonenzymatic antioxidants, namely, vitamin E or GSH [14]. Manganese-SOD detoxifies superoxide anion ($O_2^{.-}$) to give H_2O_2, which is then converted into H_2O by either GPx or catalase [14]. We have observed that GPx activity is increased in 3×Tg-AD mice compared with the nontransgenic animals, which can be a protective mechanism to neutralize the formation of H_2O_2 produced by SOD, whose activity is also increased. Because the GRd activity is not statistically altered and GPx requires GSH as a substrate, the GSH levels are decreased and the GSSG levels are increased in 3×Tg-AD mice compared with controls. Similar results were obtained in cortical tissue from Tg2576 mice, in which the activities of SOD and GPx are increased [49]. The expression of SOD as well as of the GPx is higher in AD brains than in control non-AD brains [15,50].

Vitamin E and GSH, two nonenzymatic antioxidants, were shown to be decreased in the triple-transgenic model of AD and the levels of lipid peroxidation markers, namely MDA, HNE–His protein adduct, and TBARS, were increased. In another AD mouse model, the Tg2576 mice that carry the APP Swedish mutation, lipid peroxidation also precedes apparent Aβ deposition and increases in Aβ levels [18]. However, oxidative damage in these Tg mice that harbor APP or PS1 mutations appears later than in the 3×Tg-AD mice. Accordingly, in brain tissue from PS1 M146 L mice, increased levels of oxidative stress were observed only in aged animals (19–22 months of age) [51]. In this model, vitamin E reduces lipid peroxidation, and amyloid deposition [52] and a chronic antioxidant diet can reduce hippocampal-dependent memory deficits without affecting Aβ levels or plaque deposition [53]. Vitamin E-deficient rats, which undergo continuous oxidative stress, contain dystrophic neuritis analogous to that associated with the AD senile plaques [54]. PHF are more often found in neurites with membrane abnormalities indicative of lipid peroxidation, suggesting that oxidative stress may play a role in the development of neuritic abnormalities [55]. Because we have not observed alterations, neither in enzymatic nor in nonenzymatic antioxidants, in the PS1 knock-in mouse used in this work as a control, nor in the MDA or HNE–His levels, we can suggest that the

presence of both APP and tau mutations can accelerate the appearance of oxidative stress markers. Although this hypothesis requires further investigation, it is supported by several findings. Increased tau pathology observed in aged homozygous transgenic mice harboring the P301L tau mutation was revealed in the altered lipid peroxidation levels and the up-regulation of antioxidant enzymes. Furthermore, these mice revealed an increased vulnerability of the mitochondria to Aβ insult, suggesting a synergistic action of tau and Aβ pathologies on mitochondrial function [56].

The early increase in oxidative stress can contribute to the development of NFTs in the 3×Tg-AD mice, detected at 12 months [20]. The involvement of oxidative stress and subsequent lipid peroxidation products in tau phosphorylation has been suggested [4]. Very recently, it has been demonstrated that mitochondrial oxidative stress causes hyperphosphorylation of tau in residues that are hyperphosphorylated in AD [57]. Lovell and colleagues [58] demonstrated a direct link between oxidative stress and tau phosphorylation in cortical neurons in culture. Moreover, modifications of tau by 4-HNE promote and contribute to the generation of the major conformational properties defining neurofibrillary tangles occurring in AD brains [59].

In summary (Fig. 5), using a triple-transgenic mouse model that progressively develops amyloid plaques and tangles, we demonstrated that the decrease in nonenzymatic antioxidants such as vitamin E and GSH leads to lipid peroxidation, increasing the levels of MDA, HNE–His protein adducts, and TBARS. In addition, we observed an increase in the activity of the antioxidant enzymes SOD and GPx, providing evidence that neurons mobilize antioxidants in the face of increased oxidative stress. However, GRd was not affected and thus GSH levels are further depleted, contributing to oxidative damage to nucleic acids, proteins, and lipids. This study demonstrated that in 3×Tg-AD mice the oxidative stress occurs earlier than in other previously studied transgenic animals that carry only APP (Tg2576) [18,49] or tau mutations (P301L) [56]. The obtained data support the amyloid cascade hypothesis [60], suggesting that oxidative stress is an early event in the development of AD and precedes the accumulation of Aβ in senile plaques and the formation of NFTs. Altogether, this and other studies [61] suggest that antioxidant therapy may be beneficial if given at the early stages of the AD development.

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